Research Article

# Production Performance and Humoral Immunity in IPB-D3 Chickens

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**Abstract** | This research were conducted analyzed antibodi in IPB-D3 chickens on production performance. Pedigree data and information were collected in generations 3 and 4. In the third generation, as many as 36 chickens (9 males and 27 females) aged 27 weeks and 162 IPB-D3 chicken eggs, in the fourth generation aged DOC and 2 weeks, each 81 samples. Total Immunoglobulin yolk (IgY) observations were made using Enzyme Linked Immunosorbent Assay (ELISA) and antibody titer against *Newcastle disease* virus (AbNDV) methods using Hemaglutination Inhibition (HI) tests. Descriptive analysis was performed on production performance data and humoral immunity to estimate phenotypic correlation of the statistical method of Pearson correlation analysis. The results of IgY examination in men aged 27 weeks were 9.49 ± 1.37 mg/mL and IgY in women 9.36 ± 2.89 mg/mL. AbNDV ages 0 and 2 weeks at 1.34±0.95 and 1,370.95 LOG 2 GMT. At the age of 12 weeks, the body weight of IPB-D3 chickens of G4 males is higher than that of G3 males, which is 1021.44±60.05 grams with a CV of 17.01%. Total egg production in a day in the age range of 29-40 weeks is 13.3±7.56 eggs, with a production percentage of 44.8%. Phenotypic correlation between IgY aged 0-2 with active body weight of IPB-D3 chickens aged 0 to 12 weeks showed positive but low to moderate values of 0,06-0,49. In conclusion, humoral immunity such as IgY and AbNDV did not have a significant effect on the growth and production of IPB-D3 chickens.

**Keywords** | Chicken IPB-D3, IgY, AbNDV, Body weight, Egg production, Phenotypic correlation

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## INTRODUCTION

IPB-D1 chicken is a new family of local composite chickens produced through a cross between F1 PS males (Pelung × Sentul) and F1 KB females (Native chicken × parent stock Cobb) which has been officially released by the Ministry of Agriculture of the Republic of Indonesia based on Decree No. 693/KPTS/PK.230/M/9/2019 (Al Habib *et al*., 2020). The selection of chickens is based on the fact that sentul chickens have advantages in faster weight growth, better resistance to disease, and higher egg production rates when compared to other free-range chicken breeds (Sudrajat and Isyanto, 2018). Pelung chicken as a local chicken produces good meat and the reproductive quality of Pelung chicken is very good (Junaedi and Hastuti, 2021). Native chickens have a high body resistance. The results of the study Ulupi *et al*. (2014) found native chickens showed resistance to Salmonella enteritidis in all genotypes (AG, GG, and AA) present. While broiler parent stock Cobb chickens were chosen because of their rapid growth. The results of the study Sopian *et al*. (2015) obtained chickens from Pelung-Sentul (PS)

crosses showed superior weight at DOC and 12 weeks of age (1,237.2 grams) compared to crosses between Sentul chickens and Kampung chickens (SK) (1,009 grams).

The formation of this clump was carried out with the aim of increasing the important role of local chickens, because IPB-D1 chickens have TLR4 genes (GG and AG genotypes) showing resistance to *Salmonella enteritidis* based on leukocyte count, hematological profile, and humoral immunity proven through determination of TLR4 gene by PCR and sequencing and clearance test before and after infection challenge S. enteritidis in 11 IPB-D1 chickens aged 10 weeks (Susanti *et al.*, 2020). Furthermore, IPB-D1 chickens showed a good level of immunity to the ND virus after being tested at the age of 13 weeks, both in the ND vaccinated group (n = 20) and the ND unvaccinated group (n = 10) with appropriate controls. Immunity measurement is carried out through inhibition (HI) haemagglutination test and identification of chicken white blood cell profile (SDP). Resistance and susceptibility to ND virus infection in IPB-D1 chickens are thought to be related to genetic variation (Setyaningsih *et al.*, 2020). In addition, the results of the study Al Habib *et al.* (2020) on 503 IPB-D1 chickens aged 12 weeks had an average rapid growth of 1,178 grams (females) and slaughter weights of 1,378 grams (males), when compared to the results of research by Fitra *et al.* (2023) who received a slaughter weight of 12-week-old native chickens of 1,062a±100.84 grams.

Prospective IPB-D3 chicken strains are prospective male strains of IPB-D1 chickens that have better growth (Salsabila *et al.*, 2022) however, they have not been selected for disease resistance. Despite better growth, the sustainability of breeding programs requires a thorough evaluation of the potential trade-off between superior growth and disease resistance. The purpose of the breeding program is to select genetically superior birds for meat and egg production. Maintenance, growth, reproduction, and immunity are considered as major categories in resource allocation (Siegel and Honaker, 2009). The balance between individual demands becomes crucial due to limited resources. Therefore, rapid growth selection allocates feed sources for weight gain, but the ability to respond to other demands such as immunocompetent decreases (Mohammadi-tighsiah *et al.*, 2018).

To maximize the profitability of the IPB-D3 chicken production system, it is not only important to pursue optimal growth but also take into account other aspects such as immunity. Therefore, in the selection of chickens for reproduction, it is necessary to take into account immune parameters, including humoral immune response. Evaluation of parameters such as ND (*Newcastle disease*) antibody titers and total IgY concentrations is key in understanding the chicken's immune response to disease and providing a more complete picture of the traits possessed by prospective IPB-D3 chicken strains. Thus, breeding can be directed to produce chicken strains that not only excel in growth, but also have an adequate level of resistance to diseases.

The immune system in chickens that involves the production of antibodies does play a very important role in fighting diseases such as Newcastle Disease (ND) and Salmonella sp. Antibodies produced by chickens can bind to ND virus particles and prevent them from attaching to host cells, thus helping to prevent the spread of the disease. In addition, antibodies also play a role in overcoming Salmonella sp. infection. by binding and neutralizing these bacteria. This is very important considering the high mortality rate associated with these two diseases which reaches 80% (Dharmayanti *et al.*, 2014).

The importance of keeping chickens is closely related to the degree of resistance of chickens to diseases. Therefore, the development of prospective D3 chicken strains is an effort to improve the performance of local Indonesian chickens, ensure disease resistance, adaptability to community maintenance, and rapid growth. Therefore, the purpose of this study is to support the development of IPB-D3 chickens that are more resistant to disease along with production performance.

## MATERIALS AND METHODS

Data were collected from IPB-D3 chicken populations that were randomly raised in the Field Laboratory of the Faculty of Animal Husbandry, IPB University, Indonesia. Pedigree data and information were collected in generations 3 and 4. In the third generation, a total of 36 chickens (9 males and 27 females) were randomly distributed in pairs of 1 male and 3 parent females. Each pair is transferred to a new cage (9 mating cages in total) and mated. Eggs are numbered from each cage according to the identification of breeding pairs, then put into hatching machines periodically every 4 days. The hatching machine used is an automatic hatching machine with a maintained temperature between 37-38 °C and humidity around 55%-60%. One-day-old IPB-D3 chickens are identified by wing bands and weighed immediately after hatching, then weighed monthly.

### Sampling of Parent Derived Blood and Egg Yolk

Blood sampling was carried out on IPB-D3 wing chickens (brachial veins) with a 1 mL syringe measuring 0.5-1 mL without anticoagulants that had previously been wiped with 70% alcohol. A total of 36 blood samples were obtained from the blood collection of IPB-D3 chicken elders who had reached the age of 27 weeks, as well as 3

offspring from each mother. So that the blood samples of D3 descendants at the time of DOC were 81 samples, aged 2 weeks. Blood samples are stored for approximately 24 hours and serum separated from red blood cell clots is placed in labeled microtubes, then stored at 4 °C for HI and Elisa testing.

A total of 162 IPB-D3 chicken eggs from each parent were tested in this study obtained for 3 months. The egg yolk data obtained is divided into production every month so that IgY concentration data and egg yolk ND titer are obtained 3 times. The yolk is separated from the egg white, then placed on filter paper to remove the remaining egg white. After that, the egg yolk is transferred into a microtube as much as 0.5 mL using a micropipette, then added PBS solution as much as 0.5 mL. The next step is to stir the vortex for 10 seconds, followed by centrifugation for 10 minutes at 4°C. Centrifuged supernatants are taken from microtubes using micropipettes and coded.

### Hemaglutination Inhibition (HI) Test to Measure Anti-ND Antibody Titer

The HI test or hemagglutination inhibition is a serological test in the form of inhibition of specific antibodies to the activity of ND virus antigen hemagglutination (Suharis *et al*., 2023). Antibody titer is determined based on the highest dilution inhibitory power that is still able to bind to the antigen (at a concentration of 4 HAU) and inhibit red blood cell agglutination.

Antigen and antisera positive controls were included in the test. The HI test begin by inserting a 25 µL PBS solution on a microplate added with 25 µL of sample into the first well of the microplate and serial dilution to the 11th well. ND 4 HAU antigen is added to every well, except for the 12th well. The plate is incubated at room temperature for 30-40 minutes. A total of 25 µL of 1% RBC was added and mixed into each well and incubated for 30 min at room temperature.

The assessment results if there is a deposit in the control well, the test reading begins. Antibody-positive serum is characterized by inhibition of agglutination resulting in deposition of red blood cells such as control wells. Serum titer is determined from the highest serum dilution that is still able to inhibit antigens to agglutinate chicken red blood cells. The HI test provides information on the level of immunity to ND in IPB-D3 chickens. Understanding antibody levels is critical in evaluating the immune response to ND disease, so as to support the selection of chickens that have optimal immunity for reproduction.

### Total IgY in Egg Yolk and Chicken Serum IPB-D3

Total IgY observations were conducted using the Enzyme-Linked Immunosorbent Assay (ELISA) method as reported by Ruhi *et al*. (2018). ELISA is widely applied in immunological research in poultry due to its ease and efficiency (Krzysica *et al*., 2022). In this ELISA test, egg yolk and blood serum of IPB-D3 chickens are used to determine the concentration of antibodies to the disease in IPB-D3 chickens. The first step is to dilute the Anti IgY using a buffer layer (0.05 M carbonate-bicarbonate pH 9.6) until it reaches a final concentration of 2.5 µg mL-1. The diluted antigen is introduced into all wells in the dish at the rate of 100 µL per well. The plates are incubated at 4°C overnight.

The next plate is washed 4 times using a washing buffer of Phosphate Buffered Saline with Tween (PBS-T) (PBS pH 7.4 added 0.05% Tween-20) as much as 250 µL per well. The plate is then blocked with a 5% PBS-Skim milk solution of 100 µL per well. The plate is incubated for 2 hours at 37 °C. The incubated plate, then washed as the previous step, then inserted a diluted sample (dilution 1:100) according to the pattern made and incubated 1 hour at a temperature of 37 °C. The plate is washed 4 times then anti-IgY which has been conjugated with Horse Reddish Peroxydase (HRP) (dilution 1:25000) is added to each well as much as 100 µl and incubated at 37°C for 1 hour. The plate is washed 4 times with Phosphate Buffered Saline with Tween (PBS-T) buffer and 100 µl of tetramethylbenzidine (TMB) chromogenic substrate is added to the well and allowed to stand for 20-30 minutes. The IgY concentration is read using an ELISA reader with a wavelength of 650 nm. The concentration of IgY is calculated based on the concentration value of the control IgY (Sriveny *et al*., 2006).

### Data Analysis

Descriptive analysis was carried out on production performance data and humoral immunity in D3 IPB chickens in the form of calculating the measured trait population average, standard deviation and diversity coefficient (CV). To estimate the phenotypic correlation between production performance and humoral immunity levels, we used the statistical method of Pearson correlation analysis, which allows evaluating the linear relationship between two variables.

## RESULTS

The value of IgY concentration can vary between individuals which can indicate the level of endurance of the chicken. The average value of IgY concentration and ND titer in IPB-D3 3rd generation (G3) chickens aged 27 weeks can

be seen in Table 1.

**Table 1:** IgY and AbNDV ayam IPB-D3 concentrations at 27 weeks of age

| Value | IgY (mg mL$^{-1}$) | | AbNDV (LOG 2 GMT) | |
|---|---|---|---|---|
| | male | female | Male | Female |
| Mean±SD | 9,49±1,37 | 9,36±2,89 | 0 | 1,82±1,27 |
| Min | 7,68 | 5,53 | 0 | 0 |
| Max | 11,05 | 14,55 | 0 | 4 |
| Coefficient of Variance (%) | 14,4 | 30,86 | 0 | 69,6 |

The measurement results, IgY concentration and NDV antibody titer (AbNDV) in IPB-D3 strain chickens at the age of 27 weeks can be seen in Table 1. The average concentration of IgY in males was 9.49±1.37 mg / mL, while in females it was 9.36±2.89 mg / mL. The IgY value in IPB-D3 chickens is not much different from IPB-D2 chickens which are prospective strains of IPB-D1 chickens from research Lestari *et al.* (2021) who get a total IgY of ≥ 9.55 mg / mL. The minimum and maximum values of IgY concentration in male IPB-D3 chickens at the age of 27 weeks were 7.68 mg / mL and 11.05 mg / mL, respectively, while the minimum and maximum values in females were 5.53 mg / mL and 14.55 mg / mL, respectively. This gives an idea of the range of variation in chicken immune responses at IgY concentrations. The high variability in IgY concentrations in IPB-D3 chickens is due to the main factor, namely the absence of a selection process that focuses on regulation or stabilization of IgY concentrations. In other words, the observed diversity in the chicken's immune response occurs because there has not been a selection or genetic selection that specifically leads to the regulation of IgY concentration levels. Therefore, this diversity may be influenced by genetic differences between individual chickens in populations that have not undergone specific selection related to immune responses.

The average AbNDV titer in males showed a value of 0, while females had an average titer of 1.82±1.27. This suggests a stronger immune response to Newcastle disease virus in the female group. This result is smaller than the IPB-D2 chicken study Lestari *et al.* (2021) which obtained an ND titer of ≥ 3 log 2 HI units categorized as an antibody titer that can provide protection against ND disease. IgY and AbNDV in IPB-D3 chicken egg yolks during 3 nesting periods can be seen in Table 2. Immunoglobulin Y (IgY) is a water-soluble protein found in chicken serum and egg yolks (Gaetani *et al.*, 2017).

On the analysis of the concentration of IgY and AbNDV in chicken yolks IPB-D3 G3 for 3 periods can be found in Table 2. The average IgY concentrations during the first, second, and third periods were 10.88±2.36 mg/mL, 16.31±5.68 mg/mL, and 10.51±1.83 mg/mL, respectively. The coefficients of variance for IgY concentrations in each period were 21.7%, 34.85%, and 17.41%. The average NDV antibody titer (AbNDV) in egg yolks during the first, second and third periods was 4.74±3.73 LOG 2 GMT, 5.03±4.15 LOG 2 GMT, and 4.85 ± 2.45 LOG 2 GMT, respectively. The coefficients of variance for NDV titer antibodies (AbNDV) in each period were 78.73%, 82.44%, and 50.55%. A high value of the coefficient of variance indicates a greater degree of variation in NDV titer antibodies (AbNDV). Its high diversity can actually be an opportunity, because it can be used to improve genetic quality by selection. According to Milas *et al.* (2020), diversity is very important in livestock selection because its value is very useful to see the potential diversity in the population which is the basis for the next breeding stage.

**Table 2:** Concentration of IgY and AbNDV in IPB-D3 G3 chicken egg yolk for 3 periods

| Periode | IgY | | AbNDV | |
|---|---|---|---|---|
| | Mean±SD (mg mL$^{-1}$) | Coefficient of Variance (%) | Mean±SD (LOG 2 GMT) | Coefficient of Variance (%) |
| 1 | 10,88±2,36 | 21,7 | 4,74±3,73 | 78,73 |
| 2 | 16,31±5,68 | 34,85 | 5,03±4,15 | 82,44 |
| 3 | 10,51±1,83 | 17,41 | 4,85 ± 2,45 | 50,55 |

The IgY antibodies present in the yolk (yolk plasma) act as an initial defense against infection in newly hatched chicks. They provide passive immunity to chicks during the early period of life before the chicks' own immune system develops. The concentration of IgY and AbNDV in the serum of IPB-D3 G4 chickens aged 0 and 2 weeks can be seen in Table 3.

**Table 3:** Concentration of IgY and AbNDV in serum of IPB-D3 G4 chickens aged 0 and 2 weeks

| Aged (Weeks) | IgY | | AbNDV |
|---|---|---|---|
| | Mean±SD (mg mL$^{-1}$) | Coefficient of Variance (%) | Mean±SD (LOG 2 GMT) |
| 0 | 9,92±2,74 | 27,58 | 1,34 |
| 2 | 10,41±2,88 | 27,66 | 1,37 |

The mean IgY concentrations at 0 weeks and 2 weeks were 9.92±2.74 mg/mL and 10.41±2.88 mg/mL, respectively. The coefficients of variance for IgY concentrations at both ages were 27.58% and 27.66%. In Table 3. It can be seen that the average NDV titer antibody (AbNDV) of IPB-D3 generation 4 chickens at the age of 0 and 2 weeks is 1.34 LOG 2 GMT and 1.37 LOG 2 GMT, respectively. Average body weight data (BB) of IPB-D3 generation 3

**Table 4:** Body weight of chickens IPB-D3 G3-G4

| Age (Week) | Sex | BW (gram) ± Stdev , CV G3 | BW (gr) ± Stdev , CV G4 |
|---|---|---|---|
| 0 | - | - ± - | 27,10 ± 4,41 , 16,27% |
| 4 | - | 325,39 ± 55,02 , 16,91% | 266,00 ± 48,91 , 18,39% |
| 8 | Male | 654,10 ± 96,82 , 14,80% | 521,33 ± 39,21 , 7,52% |
| | Female | 560,00 ± 109,05 , 19,47% | 369,00 ± 45,7 , 12,38% |
| 12 | Male | 894,00 ± 163,55 , 18,29% | 1021,44 ± 60,05 , 17,01% |
| | Female | 751,10 ± 182,08 , 24,24% | 684,33 ± 63,33 , 9,25% |
| 16 | Male | 1087,90 ± 158,16 , 14,54% | - ± - |
| | Female | 879,35 ± 200,59 , 22,81% | - ± - |
| 20 | Male | 1479,50 ± 139,24 , 9,41% | - ± - |
| | Female | 1044,58 ± 242,54 , 23,22% | - ± - |
| 24 | Male | 1617,90 ± 111,23 , 6,87% | - ± - |
| | Female | 1210,84 ± 240,69 , 19,88% | - ± - |

[a] Many N local chickens IPB-D3 G3 60 heads, local chickens IPB-D3 G4 83 heads; BB: Body weight (grams); Stdev: Standard deviation; CV: Coefficient of variance

**Table 5:** Mean ± elementary (KK) egg production of IPB D3 chickens aged 29-40 weeks.

| | Month to- | | | Total |
|---|---|---|---|---|
| | 1 | 2 | 3 | |
| Number of Eggs/day | 15 ± 7,03 (46,89) | 11,7 ± 7,73 (66,12) | 13,2 ± 8,27 (62,68) | 13,3 ± 7,56 (40,31) |
| Percentage (%) | 50 | 40 | 45 | 44,8 |

(G3) and generation 4 (G4) chickens at various ages can be seen in Table 4. At the age of 4 weeks, the body weight of G3 chickens reached 325.39±55.02 grams with a CV of 16.91%, while G4 chickens weighed 266.00±48.91 grams with a CV of 18.39%. At the age of 8 weeks, the G3 rooster weighs 654.10±96.82 grams with a CV of 14.80%, while the G3 hen weighs 560.00±109.05 grams with a CV of 19.47%. In G4, roosters weigh 521.33±39.21 grams with a CV of 7.52%, and hens weigh 369.00±45.7 grams with a CV of 12.38%. At the age of 12 weeks, G4 males are taller than G3 males, which is 1021.44 ±60.05 grams with a CV of 17.01%.

The average basic egg production (KK) of D3 IPB chickens in the age range of 29-40 weeks from the 1st, 2nd and 3rd months can be seen in Table 5. In the 1st month, the average egg production ± standard deviation (KK) of D3 IPB chickens was 15±7.03 eggs per day, with a production percentage of 50%. While in the 2nd month, the average egg production is 11.7 ± 7.73 eggs per day, and the percentage of production is 40%. In the 3rd month, the average egg production reached 13.2 ± 8.27 eggs per day, with a production percentage of 45%. Total egg production in a day in the age range of 29-40 weeks is 13.3±7.56 eggs, with a production percentage of 44.8%.

The correlation between the nature of IgY antibodies (yolk immunoglobulin) derived from 0-12 weeks of age with body weight (BW) from 0-12 weeks of age can be seen in Table 6.

**Table 6:** phenotypic correlation between the nature of IgY antibodies and body weight (BW) of various ages

| Trait 1 | Trait 2 | $r_p$ |
|---|---|---|
| BW0 | IgY0 | 0,066 |
| BW4 | | 0,069 |
| BW8 | | 0,49 |
| BW12 | | 0,45 |
| BW0 | IGY2 | 0,067 |
| BW4 | | 0,063 |
| BW8 | | 0,46 |
| BW12 | | 0,39 |

In this study, an analysis of the phenotypic correlation between the properties of IgY antibodies and body weight was carried out at the age of 0, 4, 8, and 12 weeks. As a result, it was found that there was a positive correlation between body weight at the age of 0, 4, 8, and 12 weeks (BW0) and IgY antibodies at the age of 0 weeks (IgY0) with correlation values of 0.066, 0.069, 0,49 and 0,45 re-

spectively. In addition, there was also a positive correlation between body weight at the age of 0, 4, 8, and 12 weeks (BW0) with IgY antibodies at the age of 2 weeks (IgY2) of 0.067, 0.063, 0.46 and 0,39, respectively.

## DISCUSSION

### IgY concentration and ND antibody titer of IPB D3 Chickens

Male and female IgY concentrations in IPB-D3 chickens showed medium category IgY concentration values, respectively of 9.49±1.37 mg $mL^{-1}$ and 9.36±2.89 mg $mL^{-1}$ (Table 1). In the opinion of Khairiyah *et al.* (2023) the value of medium category IgY concentration in local chickens ranges from 8-10 mg $mL^{-1}$. This indicates that the chicken's immune system is more responsive to the threat of certain diseases. This condition validates efforts to develop prospective IPB-D3 chicken strains to increase chicken resistance to disease. According to Xia *et al.* (2023) Immunoglobulins are widely present in the most advanced organic living bodies and provide strong immune protection to the body. Similar to immunoglobulin G (IgG) in mammals, yolk immunoglobulin (IgY) is produced by the avian immune system.

The lowest and highest minimum concentration values were found in the female IPB-D3 chicken group. According to Mohammadi-tighsiah *et al.* (2018) important factors affecting humoral immunity sex, species and race, type and concentration of antigens, and timing of immunization, inoculation frequency. Khairiyah *et al.* (2023) stated that the group of chickens that had a low IgY concentration was suspected of having an infection that caused immunodeficiency conditions. Low immunoglobulin (Ig) titers indicate humoral immunodeficiency.

The coefficient of diversity in males is smaller (14.40%) than in females (30.86%), indicating that the variation in data in males is relatively small compared to the average. In this context, data tend to be more homogeneous or less varied. Meanwhile, in females showed that the variation in data was relatively large compared to the average. In this context, data tend to be more heterogeneous or more varied.

The difference in ND abtibody titer values between individuals can be caused by non-vaccination in this study. It is indicated that the addition of this herb may not increase the titer of antibodies after vaccination. According to Arnaya *et al.* (2023) the average antibody titer in broiler chickens after vaccination days 14, 21 and 28 days is below the seropositive threshold value (< 4 HI log 2) against ND disease and ND antibody titer examination in postvaccination broilers indicates that ND vaccination in able to produce positive immunity up to broilers aged 13 days with a titer of 4.2 HI log 2.

The results showed that the total IgY concentration in IPB-D3 chicken egg yolk period 2 was higher than periods 1 and 3, which was 16.31±5.68 mg $mL^{-1}$. However, IgY concentrations in all three periods showed high category values (Table 2). This is because the IgY in egg yolks mostly comes from the IgY present in the mother's blood serum and part of the natural defense system given by the mother to her child. This is in accordance with opinion Müller *et al.* (2015) which states the transfer of IgY from serum to egg yolk is a receptor-mediated process that allows selective transfer of antibodies from parent serum. Kumaran *et al.* (2018) states that chickens can transfer a large amount of immunoglobulin from serum to their yolk, which serves as a means of passive protection against developing chicks. According to Khairiyah *et al.* (2023) Chickens that develop antibodies in response to exposure to disease agents, known as antigens, are said to have an active immune system. Meanwhile, chickens that receive antibodies from their mothers through eggs are said to have a passive immune system.

The results of the study Kurniawan *et al.* (2022) that the fastest chicken embryo death occurred in SAN eggs aged 7 days, namely 1 day after AI virus inoculation, but no hemagglutination titer was found in allantois fluid and The results showed that the highest average hemagglutination titer was 1,322.67 ± 339.73 HAU / 25 µl found in SAN eggs aged 11 days.

Table 3 presents data on IgY concentration in IPB-D3 Chicken serum in the G4 group at 0 and 2 weeks of age. At 0 weeks of age, the mean IgY concentration was 9.92 mg/mL, with variation measured through a standard deviation of 2.74 mg/mL. A diversity coefficient of 27.58% indicates a significant degree of variation between individuals in the group at this early stage. When reaching 2 weeks of age, the average IgY concentration increased to 10.41 mg/mL, and the standard deviation increased slightly to 2.88 mg/mL. Despite the increase, the diversity coefficient remained relatively stable at 27.66%. This suggests that, despite changes in IgY concentration levels, variation between individuals within the group remains significant at this stage.

Passively acquired maternal antibodies can inhibit the formation of immunoglobulins, thus affecting the success of vaccination. Vaccination done when maternal antibodies are still in the blood circulation will be useless, because it will be neutralized by maternal antibodies. This antibody neutralization reaction will cause a decrease in antibody titer in the broiler body (Nurkholis *et al.*, 2014).

According to Bagus *et al.* (2023) changes in *Newcastle disease* antibody titers in broilers decreased significantly from 7 days to 14 days old and decreased again from 14 days to 21 days old. According to (Touko *et al.*, 2015) antibodies from the brood transferred through the blood effectively protect chicks after 10 days of hatching. Due to a decrease in maternal antibody titer and becoming no longer positive at the age of 14 days, it is necessary to carry out a vaccination program to prevent *Newcastle disease.*

Antibodies when the child just hatches are called maternal antibodies, which is at the age of 0-2 weeks. The maternal phase of IgY antibodies in chicks illustrates the importance of the hen's role in providing early immune protection to newly hatched chicks, helping them to cope with the risk of infection and ensuring early health in their growth phase. Maternal antibodies that are still present in the body of chicks will affect the formation of immunity, because maternal antibodies can neutralize the virus and vaccine agents that enter, thereby reducing the stimulation of active immunity formation.

## Production performance of IPB D3 Generation 3 and 4 Chickens

IPB D3 G3 chickens intended as G4 parents have an average body weight of 1087.90 g in males and 879.35 g in females when chickens are 16 weeks or 4 months old (Table 4). This is smaller than the study Habiburahman *et al.* (2018) which states that IPB-D1 chickens have a body weight of 1.2 kg at the age of 12 weeks. This is possible for differences in maintenance management, the environment and the feed provided Dameanti *et al.* (2020). IPB-D3 G3 chickens have an average body weight of 894 g in males and 751 g in females (Table 4). The things obtained are not much different from research Salsabila *et al.* (2022) which states that the body weight of male IPB-D3 chickens reaches 883 g. This is possible that the research carried out has the same management and environment, namely in the Sukabumi area of Central Jampang. Trianty *et al.* (2022) mentioned that environmental and feed factors greatly affect the body weight and posture of native chickens.

IPB-D3 Generation 4 chickens have an average body weight of 975.61 g – 1035.66 g in males and 684.33 g – 747.66 g in females aged 12 weeks (Table 4). This is different from the body weight of IPB D3-G3 chickens (their parents). IPB-D3 G3 chickens have different places of maintenance, this can be the main factor in the body weight of different G4 and G3. Noor (2008) Mention of genetic factors can be influenced by the environment. IPB-D3 G4 chickens are raised from DOC until 3 months old in the Field Laboratory of Bogor Agricultural A) with Universi more controlled temperature, humidity and feeding situations. This indicates that external factors such as management, environment and feed given to local chickens IPB-D3, G3 and G4 have a very significant influence on the body weight they will have.

Local chicken egg production is low, one of which is native chicken at the age of 23 weeks produced 26 eggs / 8 weeks (46.43%). Low egg production of local chickens due to genetic factors and feed. Egg production obtained from D3 IPB chickens can be seen in Table 5, which is 44.8% lower than the literature and also lower than IPB-D1 G7 chickens from the research of Habiburahman *et al.* (2020) which reached 49.22% production. The low egg production of IPB D3 chickens caused by low protein in feed and early production period. The egg production of IPB D3 chickens is observed for 3 months. It can be seen in Table 5, egg production in the first month reaches 50%, in the second month it decreases with 40% production and in the third month egg production reaches 45%.

The coefficient of diversity of egg production of IPB D3 chickens is still diverse, it can be seen in Table 5. The value of the diversity coefficient each month ranges from 46.89-66.12, with the diversity coefficient in total production having a value of 40.31. The high coefficient of diversity is due to the egg production of each individual IPB D3 chicken is still very diverse. The egg production of IPB D3 chickens for 90 days was studied at least 5 eggs and at most 65 eggs, this is what causes the high value of the diversity coefficient. Low egg production in some individuals is due to the appearance of incubation and some parents who are stressed due to overly aggressive males. Aggressiveness in roosters is because IPB D3 chickens are formed from 75% of genetics derived from local chickens. High levels of aggressiveness are also likely caused by the high dopamine content in IPB D3 chickens. Dopamine itself is a compound that plays an important role in regulating emotional levels (aggressive). Increasing egg productivity is important to increase production efficiency. In this study, it is expected to obtain basic data to determine the selection of chicken breeds that have good egg production, so that IPB D3 chickens are expected to have high egg production in the future.

The productivity of IPB-D3 chickens in the G3 and G4 generations in this study showed levels below optimal but still in good condition. In addition, it was found that humoral immunity such as IgY was also categorized as moderate. This can be interpreted as an indication that the level of immunity is able to provide protection against disease. Thus, the increased level of antibodies contributes to the fact that the productivity of IPB-D3 chickens is not at the optimal level or above it. Van Der Most *et al.* (2011) state that productivity including growth and egg production becomes weak as the immune system improves.

### Estimation of phenotypic correlation between antibodies and body weight (BW)

Phenotypic correlation between IgY aged 0-2 weeks with body weight in IPB-D3 chickens aged 0 to 12 weeks showed positive values but low to moderate. This is because IPB-D3 chickens are selected in the direction of growth so they have not been selected for their humoral immune response like IgY. This is supported by the opinion Mohammadi-tighsiah *et al*. (2018) selection for humoral immune response does not adversely affect the growth performance of ungas.

The body's defense system, including humoral immune responses such as IgY production, plays an important role in fighting disease agents such as bacteria, viruses, parasites, and fungi in IPB-D3 chickens. The relationship between IgY levels and chicken growth can be interpreted as a reflection of the balanced immune response and growth. Although the observed correlation showed low to moderate positive values, these results provide important insights regarding the integration between immunological and growth aspects in the development of IPB-D3 chickens that are more resistant to disease, as well as their production performance. The implications of these findings may support further understanding of the complexity of interactions between immune responses and chicken growth characteristics in the context of developing superior avian strains. This is in accordance with the statement Khairiyah *et al*. (2023) that the body's defense system has an important role in fighting disease agents such as bacteria, viruses, parasites, fungi, and others which can result in decreased productivity and even death in livestock.

## CONCLUSION

Humoral immunity such as IgY and AbNDV do not have a significant effect on the growth and production of IPB-D3 chickens. This is due to the fact that IPB-D3 chickens are prospective chicken strains that have not undergone selection based on their antibody titers. Nevertheless, the development of IPB-D3 chickens has the potential to improve resilience and production performance, underscoring the importance of understanding the balance between immune response and growth in local chicken breeding efforts. This conclusion supports efforts to develop more resilient IPB-D3 chickens, with the hope that it can provide benefits for livestock productivity and the welfare of farmers in Indonesia.

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## ETHICAL APPROVAL AND INFORMED CONSENT

Research is carried out in accordance with the principles of animal welfare. The protocol has been approved by a team of research ethics commission, Faculty of Medicine, Andalas University. The number of passing the ethical review is: 19/UN.16.2/KEP-FK/2024.

## CONFLICT OF INTEREST

The authors have declared no conflict of interest.

## NOVELTY STATEMENT

In this study, we explored humoral immune response and production performance in IPB-D3 chickens of the third generation and its derivatives, the fourth generation. We sought to understand whether the immune qualities and productivity of its production were passed on to the offspring. Our findings provide a clear picture of whether the immune system correlates with better production performance in IPB-D3 chickens, opening the door to further understanding of the complex relationship between the two factors in the context of local chicken breeding.

## AUTHORS CONTRIBUTION

Nadya Indriyani acted as the main author of the manuscript and conducted research related to production performance analysis and humoral immune analysis in the form of IgY. Sri Murtini was responsible for humoral immune tests in the form of IgY and ND titers. Sri Darwati contributed to the analysis of production performance. Cece Sumantri analyzed the inheritance in immune properties and production performance.

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